Comprehensive extractables and leachables characterization through an integrated single injection Quan/Qual analysis using a benchtop multi-reflecting time-of-flight mass spectrometer

Authors: Rachel Sanig[1], Lee A. Gethings[1], Jayne Kirk[1], Richard Lock[1], Shashank Jain[2]
Affiliations: [1]Waters Corporation, UK [2]Waters Corporation, US

Introduction

When screening for extractables and leachables (E&L) from pharmaceutical packaging and medical devices,[1] it is critical to identify all extractables found at levels above the analytical evaluation threshold for toxicological assessment and to quantify low levels of leachable compounds.[2]

An extractables screening workflow was reported previously using a benchtop multi-reflecting time-of-flight mass spectrometer (MRT MS) for consistent low- to sub-ppm mass accuracy, allowing for increased extractables identification confidence.[3] Once extractables are identified a leachables experiment needs to be undertaken, this can be undertaken on the same platform with a highly sensitive Tof MRM analysis.

Here we report a combined analysis approach on one benchtop MRT MS. A time-of-flight multiple reaction monitoring (Tof MRM) mode for targeted quantitation was acquired simultaneously with a data independent acquisition (DIA) mode for confident extractables screening. This dual acquisition schema supports quantitation of known leachables and concurrent monitoring of potential new extractables through screening and identification of unknowns.

Figure 1. Waters Xevo™ MRT P10 Mass Spectrometer and ACQUITY™ Premier System

Experimental

A standard mix (E&L SST) containing typical extractables was injected. Eluate was analyzed using an advanced mixed mode acquisition incorporating, in parallel, targeted and untargeted acquisitions. (DIA scans (MS^E) with scheduled targeted Tof MRM within a single injection.) In addition, a nasal spray solution was spiked with the E&L SST mix to investigate the results in a complex matrix.

LC CONDITIONS: ACQUITY Premier System	
Column	ACQUITY CORTECS™ C18, 90 Å (1.6 µm, 2.1 x 100 mm)
Mobile Phase A / B	Water + 1 mM ammonium acetate + 0.1% formic acid / Methanol
Flow Rate	0.3 mL/min
Column Temp.	50 ºC
Injection vol.	1 µL
Gradient	15 minutes

MS CONDITIONS: Xevo MRT P10 MS	
Acquisition range	*m/z* 50-1200
MS^E Scan Speed	10 Hz
MRM dwell time	Optimized per MRM
Source/Desolvation temp	120 ºC / 550 ºC
Desolvation/Cone gas flow	800 / 50 L/hr
Column Temp.	ESI+ 2.5 kV ESI- 1.5 kV
Collision energy	Low: 6 eV High: 20-60 eV

Data Management
The waters_connect™ Software Platform was used for data acquisition and the UNIFI™ Application and MS Quan application out of the waters_connect Software Platform were used for data processing.

Results

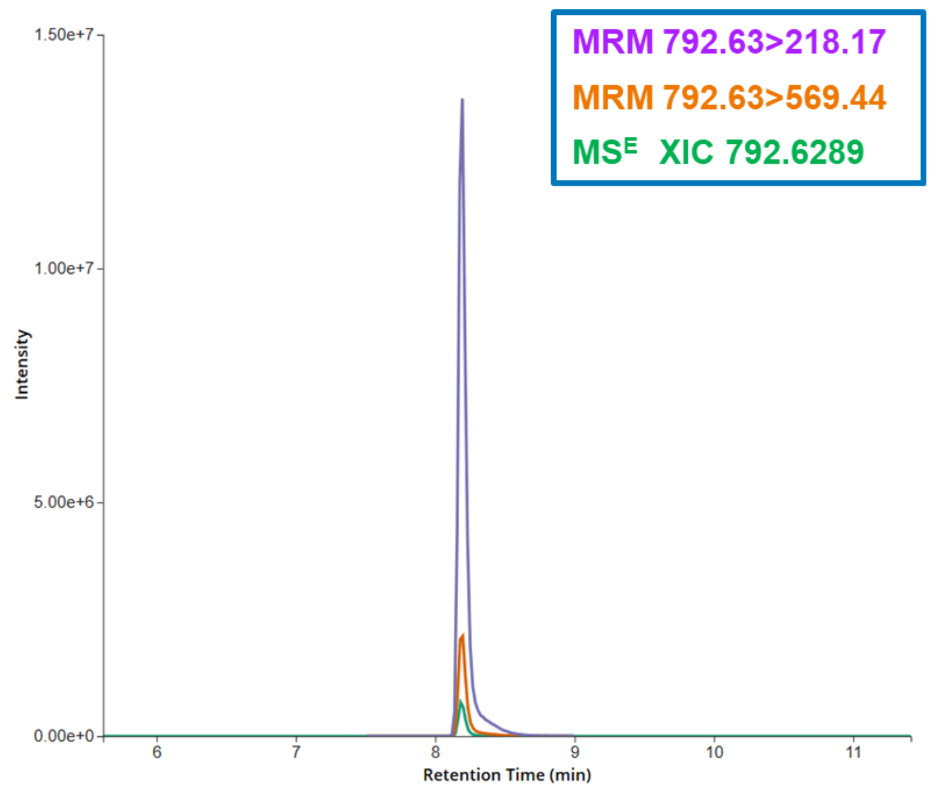

Figure 2. Overlaid chromatograms of MS^E and two MRM transitions acquired in the same injection for Ethanox 1330.

Tof MRM utilizes an Enhanced Duty Cycle (EDC) mode, where target ions are trapped and released with timing synchronization with the pusher. Ion utilization over a specific *m/z* range approaches 100%, increasing the sensitivity of the assay.[4]

MS^E is a data independent approach where alternating low collision and high collision energy is applied, enabling the acquisition of both precursor and fragment ions throughout the entire chromatographic run.[5]

Combining these two modes in one injection allows for highly sensitive quantitation whilst enabling retrospective extractables screening. Figure 2 shows the sensitivity gains with the MRM transitions compared to the XIC of Ethanox™ 1330 from MS^E mode acquired in the same injection.

Optimized Tof MRM transitions were acquired to quantify E&L compounds at trace levels. The propylparaben calibration curve, in negative ionization mode, was linear from 0.01 ng/mL (S/N 23) to 100 ng/mL (R^2 0.99). An average of the concentrations of the replicate spiked compound in the sample was calculated to be 10.04 ng/mL (actual concentration 10 ng/mL). (Figure 3)

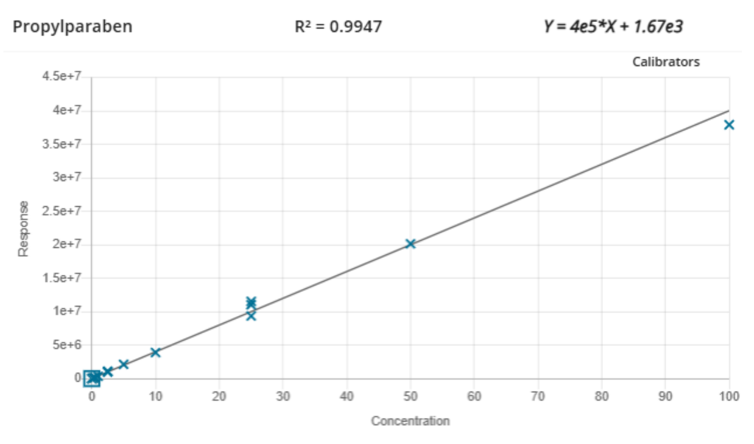

Figure 3. Propylparaben calibration curve.

Compound Name	Injection Name	Response	Calculated Concentration (ng/mL)	Expected Concentration (ng/mL)	% Deviation	Signal to Noise
Propylparaben	MeOH		Not Detected		No level	
Propylparaben	MeOH		Not Detected		No level	
Propylparaben	C1	9727	0.01	0.01	4.33	24
Propylparaben	C2	22830	0.04	0.05	-11.23	23
Propylparaben	C3	39139	0.09	0.1	-13.35	45
Propylparaben	C4	94486	0.23	0.25	-7.98	79
Propylparaben	C5	168754	0.42	0.5	-15.5	132
Propylparaben	C6	353783	0.90	1	-9.81	474
Propylparaben	C7	984017	2.53	2.5	1.4	176
Propylparaben	C8	2140121	5.53	5	10.61	190
Propylparaben	C9	3896294	10.10	10	0.81	206
Propylparaben	C10	9368267	24.30	25	-2.96	191
Propylparaben	C11	20134730	52.20	50	4.32	204
Propylparaben	C12	37927199	98.30	100	-1.74	212
Propylparaben	IPA	10757	0.01			12
Propylparaben	IPA	6813	0.00			14
Propylparaben	IPA	5853	0.00			16
Propylparaben	IPA spiked	3999580	10.30	10		215
Propylparaben	IPA spiked	3773181	9.76	10		205
Propylparaben	IPA spiked	3659393	9.47	10		204
Propylparaben	N50 spiked	3550718	9.19	10		209
Propylparaben	N50 spiked	3781450	9.78	10		201
Propylparaben	N50 spiked	4452750	11.50	10		222

Table 1. Calculated concentration of propylparaben in the spiked sample.

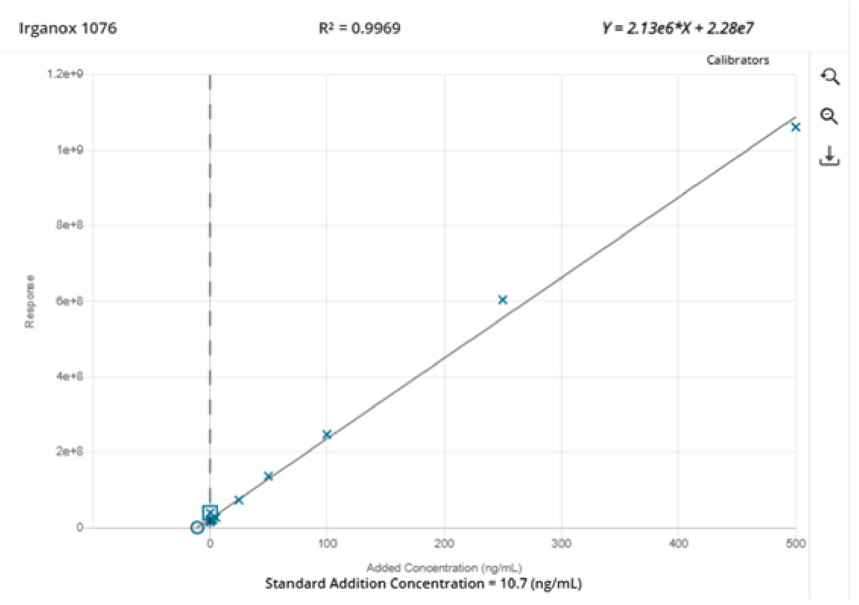

Figure 4. Standard addition calibration curve for Irganox 1076.

Using the software, a standard addition approach was utilized for any compounds that are present in the blanks. For example, for Irganox™ 1076, the spiked concentration in the samples was calculated to be 10.7 ng/mL (actual concentration 10 ng/mL). (Figure 4)

The MS^E data, acquired during the same experiment, were screened against the Waters E&L library for identification. The spiked standards were confidently identified in addition to other library matches, including erucamide (Figure 5). Data generated in this study provided mass accuracies with an RMS of low- to sub-ppm. This significantly narrows the window of possible compounds and vastly increases the confidence in the identifications returned.

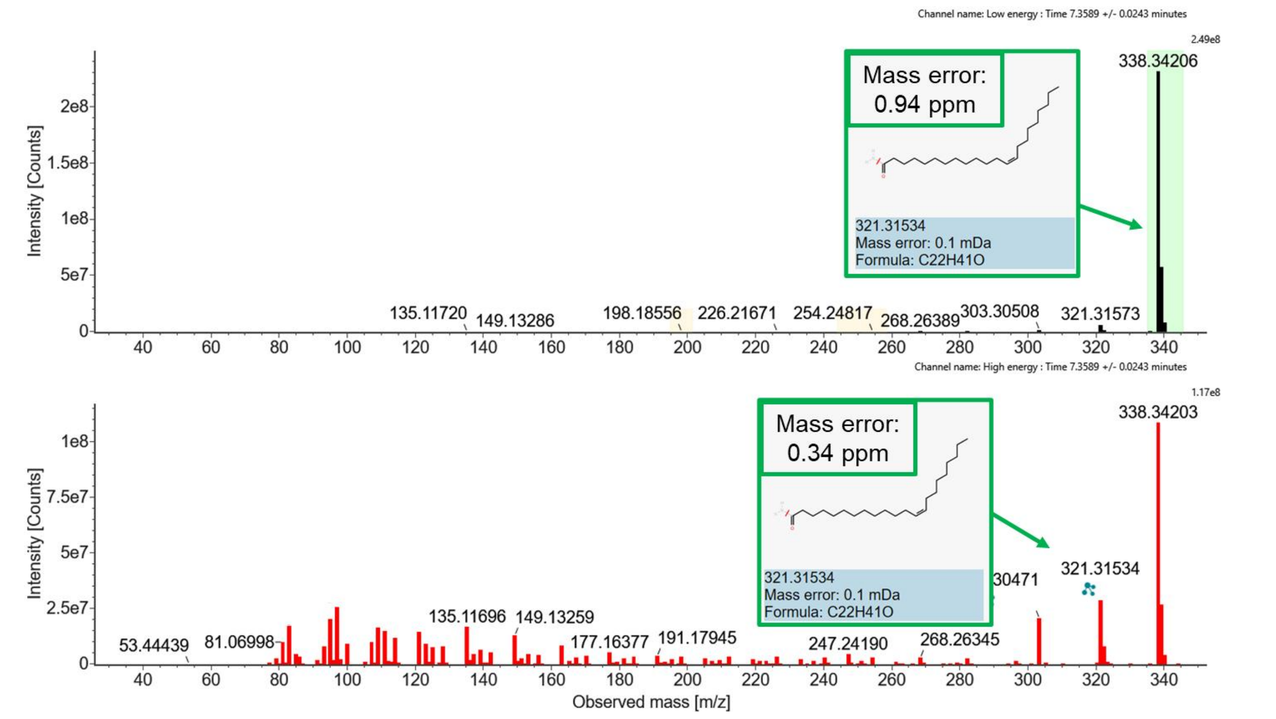

Figure 5. Putative identification of protonated m/z 338.3417 (mass error 0.94 ppm) as erucamide from MS^E. Mass fragment 321.3152 m/z (mass error 0.34 ppm).

Conclusions

- **Advanced mixed mode acquisition of quan/qual data in one injection to address a real workflow bottleneck in E&L analysis.**
- **Targeted leachables quantitative analysis with a highly sensitive Tof MRM mode.**
- **Concurrent MS^E mode acquisition for retrospective data interrogation with high mass accuracy precursor and fragment ion data for confident identifications.**

References

1. USP-NF/PF, <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. https://doi.usp.org/USPNF/USPNF_M7127_03_01.html
2. ISO 10993-18:2020 Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process, https://www.iso.org/standard/64750.html
3. Sanig R., Kirk J., Gethings L., Lock R. Increased Identification Confidence for Extractables Screening Using the Xevo™ MRT Mass Spectrometer. Waters Application Note 720008970. August 2025.
4. Daly M., Gethings L., Hughes C., Lock R., Syed N. Tof MRM for the Quantification of Peptide Biomarkers in Human Glioblastoma with the Xevo™ MRT Mass Spectrometer. Waters Application Note 720008972, October 2025.
5. Stevens D., Cabovska B., Bailey A. Detection and Identification of Extractable Compounds from Polymers. Waters Application Note 720004211. January 2012.

 720009426EN